

A STUDY OF THE EFFECTS OF SIZE-DEPENDENT  
PROCESSES ON SURVIVAL AND GROWTH OF  
ATLANTIC COD (*Gadus morhua*) LARVAE

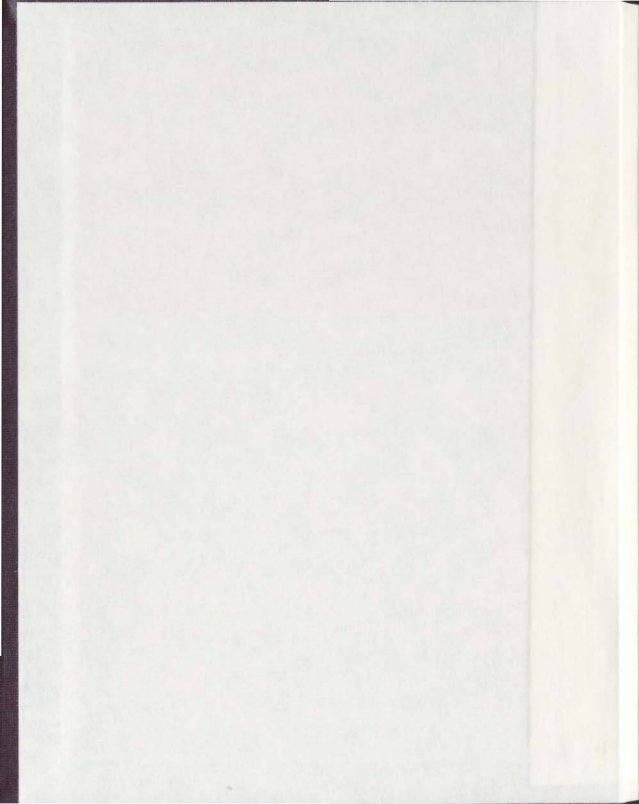
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**A study of the effects of size-dependent processes  
on survival and growth of Atlantic cod (*Gadus  
morhua*) larvae**

by

©Yingming Zhao

**A thesis submitted to the School of Graduate Studies  
in partial fulfillment of  
the requirements for the degree of  
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## **Abstract**

Fish year-class strength can be established at early life stages, such as the egg and larval stage. A small variation in growth and survival during these early life periods can result in a substantial variation in fish recruitment. Therefore, a better understanding of factors influencing growth and survival of fish eggs and larvae can help fisheries scientists better understand the variations in fish population sizes. Based on a literature review and laboratory experiments, this study investigated the size-dependent effects on early life stages (egg and larvae) of Atlantic cod (*Gadus morhua*).

Egg size can be influenced by many factors including female size (age, length or weight), fecundity and seasonal temperature. Larval size at hatching is often related to egg size and incubation temperature. Size (stage)-dependent survival has been observed for larvae in many studies. Growth rate, which may be influenced by many factors including temperature and food supply, is one of the key factors determining larval size and mortality rate.

For Atlantic cod, my study showed that larger eggs yielded larger larvae at hatching, but took longer to hatch. Larval size at hatching and incubation time were negatively correlated with incubation temperature. Although neither egg size nor incubation temperature was found to affect yolk size at hatching, higher accumulated incubation temperature significantly decreased the yolk size at hatching, but increased larval size at hatching.

The larval survival and growth experiment showed that feeding conditions and larval size at hatching significantly influenced larval survival. Better feeding resulted in higher survival. The study found that the survival rate for small larvae was higher than that for large larvae, which might result from the absence of predators in this study. Higher temperature reduced the time of yolk utilization and thus caused the cod larvae to start exogenous feeding earlier. The growth rate of cod larvae during the exogenous feeding period is higher than that during endogenous feeding period. The first few days of growth mainly resulted in a significant increase in larval weight. Delayed first feeding significantly decreased the growth rate in cod larvae. However, the large larvae showed a higher growth rate compared with small larvae under the delayed first feeding condition. After a 10 to 13-day acclimatization, the larvae under delayed first feeding exhibited the “compensatory growth”.

The size effect on cod larval growth was only significant in the delayed feeding condition, which implies that “the bigger the better” is more evident in cod larvae under unfavourable conditions, such as delayed initial feeding in this study.

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# **Chapter 1: General Introduction and Overview**

## **1.1 Introduction**

For those who study fish early life history, egg and larval size are two important parameters to be explored. Their effects on fish larval growth and survival and thus recruitment to the juvenile or adult stage have long been studied by fisheries scientists. The focus of this thesis is to evaluate the impacts of egg and larval sizes on survival and growth of Atlantic cod (*Gadus morhua*).

The thesis is composed of four chapters. The first chapter (i.e. the present chapter) is based on a literature review covering more than 100 fish species from freshwater, marine and brackish habitats. This chapter addresses the following questions:

- (1) What causes the size differentiation in fish eggs and larvae?
- (2) What effects can such differentiation impose on larval growth and survival?
- (3) Why is Atlantic cod used as the target species in the study?

In Chapter II and III, two experiments were set up to test the following hypotheses:

- (1) Egg size and incubation temperature influence larval size at hatching, yolk size, and larval incubation time of Atlantic cod.
- (2) Larval size at hatching which is related to egg size, can influence larval growth and survival in Atlantic cod.

- (3) Resistance to starvation for first feeding Atlantic cod larvae is size-dependent.

In Chapter 4, a general summary is presented and the problems which need to be addressed in future studies are discussed.

## **1.2 What factors can influence difference in fish egg and larval size?**

Maternal effects are the first consideration in the study of offspring size. Age, condition and length or weight are some of the primary factors that affect the maturation and reproductive characteristics of female fish. For many fish species, one or more of these parameters has been shown to positively relate to egg size (e.g. Chambers & Leggett, 1996; Chambers & Waiwood, 1996; Petersson et al., 1996; Marteinsdottir & Steinarsson, 1998; Benoit & Pepin, 1999; Heath et al., 1999; Hendry et al., 1999; Morita et al., 1999; Olofsson & Mosegaard, 1999; Vallin & Nissling, 2000). Female age or size-specific fecundity is negatively related to egg size in brown trout (*Salmo trutta*) (Lobon-Cervie et al., 1997; Olofsson & Mosegaard, 1999), Chinook salmon (*Oncorhynchus tshawytscha*) (Heath et al., 1999), sockeye salmon (*Oncorhynchus nerka*) (Quinn et al., 1995), chum salmon (*Oncorhynchus keta*) (Hayashizaki, 1995), blueback herring (*Alosa aestivalis*) (Jessop, 1993), spring-spawning herring (*Clupea harengus*) (Winters et al., 1993), Japanese anchovy (*Engraulis japonicus*) (Imai et al., 1997) and other fish species (Cambray & Bruton, 1994; Hopkins et al., 1995; Vacchi, 1996). Therefore, maternal conditions significantly influence their offspring size in some fish species.

For those species whose spawning is protracted over several months or seasons, such as Atlantic cod (*Gadus morhua*), Japanese flounder (*Paralichthys olivaceus*), sole (*Solea solea*), herring (*Clupea harengus membras*) and captive turbot (*Scophthalmus maximus*), egg size has been shown to significantly decline during the course of spawning (McEvoy et al., 1993; Rajasilta et al., 1993; Rijnsdorp & Vingerhoed, 1994; Mihelakakis et al., 1995). It has been suggested that temperature can also have a significant influence on the egg size of some fish species although the underlying mechanism remains unclear (Mihelakakis et al., 1995; Miller et al., 1995; Sarvala & Helminen, 1995). Thus, the seasonal or environmental effect is significant in differentiating egg size of some fish species.

Many researchers have identified positive correlations between larval size at hatching and egg size (e.g. McEvoy & McEvoy, 1991; Marteinsdottir & Able, 1992; Araujo-Lima, 1994; Baroudy & Elliott, 1994; Iguchi & Yamaguchi, 1994; Ferguson et al., 1995; Hayashizaki et al., 1995; Chambers & Leggett, 1996; Kristjansson & Vollestad, 1996; Pepin et al., 1997; Imai & Tanaka, 1997; Christiansen et al., 1998; Marteinsdottir & Steinarsson, 1998; Trippel, 1998; Huang et al., 1999; Gisbert et al., 2000; Teather et al., 2000; Voellestad & Lillehammer, 2000). Large eggs are also likely to yield the large yolk-sac larvae at hatching in some fish species (McEvoy & McEvoy, 1991; Kristjansson & Vollestad, 1996; Trippel, 1998; Gisbert et al., 2000; Voellestad & Lillehammer, 2000). Pepin et al. (1997) showed that increasing temperature resulted in an increase in larval size at hatch in Atlantic cod. In at least one fish species, California killifish (*Fundulus parvipinnis*), salinity levels during incubation or fertilization were found to influence



larval size at hatching (Rao, 1974). Therefore, the larval size at hatching can be influenced by both biotic and abiotic factors.

The growth rate during the larval stage obviously has a direct effect on larval size and the time and size at metamorphosis (Houde, 1986; Bertram et al., 1997; Exadactylos et al., 1999). Larval growth rate exhibits temperature- and density-dependent effects, i.e. fish larvae in high density and low temperature grew slower than those in low density and high temperature due to competition for food and space and temperature-dependent metabolism effects (Jenkins et al., 1991; Agnese et al., 1995; Campana, 1996; Daniels et al., 1996; Dulcic, 1997; Leising & Franks, 1999; Partridge & DeVries, 1999; Rose et al., 1999). Thus, availability of food suitable for fish larvae is one of the main factors in determining the growth rate, and plays an important role in affecting larval size (Brown & Taylor, 1992; Verreth et al., 1993; Gotceitas et al., 1996). Therefore, any factor which significantly affects larval growth rate influences the fish larval size at age. In Pacific herring, Wespestad & Moksness (1990) observed that larval size frequency from different females, unimodal at hatching, was separated into three modes after two weeks of growth within a uniform environment and these modes persisted until the juvenile stage. This pattern suggests that the female or egg characteristics can affect larval size by genetically influencing the growth rate (Monteleone & Houde, 1990).

In the field, the mean size of a cohort is often used as to illustrate the growth of larvae. Cowan et al. (1996) found that size-selective mortality, which resulted in high survival rate for large larvae, tended to shift the mean larval size of a cohort towards a large value.

### **1.3 What are the effects of size differences in fish eggs and larvae on their growth and survival?**

There are several studies which suggest that large eggs have higher survival and hatch rates in fish (Matrteinsdottir & Able, 1992; James et al., 1997). Both field observations and numerical simulation studies have demonstrated size-selective mortality as a factor influencing egg predation survival, i.e. large eggs experience lower mortality from predation (Ware, 1975; Rijnsdorp & Jaworski, 1990; Rijnsdorp & Vingerhoed, 1994; Wieland & Koester, 1996). However, Wieland & Koester (1996) showed that predation mortality for fish eggs was dependent on the state of development. Eggs in an advanced stage of development suffered higher predation mortality due to an increase in visibility caused by pigmentation of the embryo. By influencing the egg buoyancy and thus hatching location (depth) where adequate oxygen concentration is guaranteed, egg size significantly influences hatch rate and survival (Nissling et al., 1994; Nissling & Vallin, 1996; Vallin & Nissling, 2000). Therefore, egg size plays a significant role in egg survival and hatching.

Egg size can directly influence larval survival and growth. For some fish species, egg size is positively related to size of yolk at hatching which is an index of the amount of nutritional resource available to the larvae for endogenous feeding following hatch, and thus could influence larval growth and survival before exogenous feeding starts

(McEvoy & McEvoy, 1991; Kristjansson & Vollestad, 1996; Trippel, 1998; Huang et al., 1999; Gisbert et al., 2000; Voellestad & Lillehammer, 2000).

Like egg size, larval size also significantly affects an individual's growth and survival potential. As larvae increase in size, they are able to swim faster and have better visual acuity, thus they have greater ability to catch prey and avoid predators. Therefore, large larvae grow better and experience lower mortality than small larvae (Bagarinao et al., 1986; Margulies, 1989, 1990; Cowan & Houde, 1992; Cowan et al., 1996; William et al., 1996; Fiksen et al., 1998). For fish larvae, mouth gape places an upper limit on prey size (Timmerman et al., 2000). For a given fish species, large larva has a large mouth gape, which increases the size range of their potential prey and lessens starvation resulting from prey of unsuitable sizes (Hunter & Kimbrell, 1981; Armstrong et al., 1998; Davis & Todd, 1998). Hickey (1979) showed in herring and salmon that, comparing to the smaller larvae, large larvae had a greater ability to recovery from mechanical injuries. Therefore, many factors important to larval survival and growth were regarded as size-dependent (Miller et al., 1988).

#### **1.4 Why to use Atlantic cod as a target species in my study?**

Cod in the Northwest Atlantic are managed as twelve stocks (DFO 2000). Among them, the northern (NAFO division 2J3KL) cod stocks have been one of the largest stocks in the world (DFO 2001). Cod stocks around Newfoundland, Canada, have been exploited for nearly 500 years (Lear, 1998) and provided the livelihood for over 40,000

commercial fishermen of the island (Trippel, 1995). The cod fishery is of great importance economically and socially to the Province of Newfoundland and Labrador, and cod has been called the “Newfoundland currency” (DFO, 1999).

The dramatic decline in stock size in several cod populations caused the collapse of commercial fisheries throughout Atlantic Canada provinces in the early 1990s, which forced many fisherman to abandon their traditional livelihood (Baird et al., 1992; Sinclair, 1993; Trippel, 1995). Confronting such economic and social disaster from the collapse, the Canadian government imposed fishing moratoria on six Atlantic cod stocks from 1992 to 1994 with the purpose of bringing the stocks back to historical level. According to the compensatory hypothesis which is widely tested and supported as the population-dynamics regulatory mechanism in fishery science, the effects of overfishing are reversible and the time of recovery of a collapsed fish stock should be predictable (Myers et al., 1995; Myers et al., 1997). For the northern cod stock, 7 years of moratorium on commercial fishing should triple its stock size (Myers et al., 1997; Shelton & Healey, 1999). After nearly a decade of closure of the commercial fishery, however, there have been few signs of recovery in stock size, which was termed a “recruitment dilemma” by the Fisheries Research Conservation Council (FRCC, 1999). One of the hypotheses to explain the dilemma is that the stocks are experiencing a higher mortality during their earlier life stages, which causes a “recruitment-failure” (de Young & Rose, 1993; Atkinson & Bennett, 1994; Mann & Drinkwater, 1994; Sinclair, 1994).

Along with the decline in cod stock size, the structure of the cod spawning stock biomass has exhibited declines in size (both length and weight) at age as well as size or

age at maturity (Trippel et al., 1997; Chen & Mello, 1999), which has resulted in lower reproduction potential of the stock and more small eggs and larvae, as well (Hutchings & Myers, 1993; Trippel 1995, Trippel et al 1997 a, b, Trippel, 1998). Many researchers have explored the effects of egg and larval size on survival and growth in Atlantic cod. Pepin et al (1997) studied the impacts of egg size and hatching temperature on larvae size at hatching in Atlantic cod. They showed that egg size and temperature both were positively related to larvae size at hatching. However, they did not investigate the implications for larval yolk size and effects of size at hatching on larval survival and growth. Trippel (1998) showed that the female spawning history and egg size significantly influenced the yolk size of cod larvae under constant temperature. Puvanendran and Brown (1999) demonstrated that prey concentration caused significant difference in cod larval survival and growth, but they ignored larval size effects. Although Marteinsdottir and Steinarsson (1998) showed a positive relationship between egg size and some parameters related to larval viability of Atlantic cod, such as the age at first feeding, successful development of a swimbladder and growth rate, the results were based on female or batch level and prey density was constant, i.e. 500 prey L<sup>-1</sup>.

The diverse approaches and analyses from these studies leave many questions unanswered about the potential consequences of changes in egg size on the reproductive potential of cod stocks. The objective of this thesis is to (1) test the effects of egg size and temperature on the larval size and yolk size at hatching and (2) explore what will be the effects of egg size and larval size within a batch on survival and growth under different feeding condition in Atlantic cod larvae.

## **Chapter 2: Impacts of egg size, hatching temperature on size at hatching, yolk size at hatching and incubation time in Atlantic cod**

### **2.1 Introduction**

Fish year-class strength can be established at very early life stages, such as the egg and larval stages (Bailey & Houde, 1989). The survival and growth of fish at these life stages can greatly influence the number or biomass of recruits to population, and thus the sustainability of the fishery. Small variations in these parameters among years can result in large fluctuations in recruitment. It is, therefore, important to identify and understand the factors that may influence the growth and survival of larval fish.

Egg quality is one of many factors that can influence the survival and development of fish eggs. However, because egg quality varies among species and our knowledge on the effects of these factors is limited, there is little agreement regarding a general index that can be used to assess egg quality (Brook et al., 1997). In some studies, buoyancy has been used as an indicator of egg quality (McEvoy, 1984; Kjorsvik et al., 1990). Other features, such as appearance and development of larvae, have also been used as indicators (Bromage et al., 1994; Kjorsvik, 1994). Although there is little evidence showing that egg size alone is an adequate indicator of egg quality for many fish species (Springate & Bromage, 1985; Bromage et al., 1992), it has been found that egg size is positively related to larval size at hatch and future larval survival and growth (Blaxter & Hempel, 1963; Knutsen & Tilseth, 1985; Marteinsdottir & Able, 1992; Miller

et al., 1995). Moodie et al. (1989) showed that egg size was positively correlated with yolk and oil volume in walleye. The larvae from larger eggs tended to be significantly larger, and their survival, growth and feeding were better compared with those from small eggs. Rijnsdorp and Jaworski (1990) used survey data and mathematical models to demonstrate that egg survivorship is size-dependent in plaice and cod, concluding that egg mortality rate decreased as egg size increased.

For fish larvae, growth and survival both tend to be size-dependent (Beyer, 1989; Pepin, 1993; Pope et al., 1994; Houde, 1997; Otterlei et al., 1999). Larval size at hatching is an important factor in determining their subsequent survival, growth and feeding behavior. An increase in larvae size at hatching increased the flexibility in time to exogenous feeding and prolonged the time between the larval first feeding and a "point of no return" during which fish larvae can find suitable prey (Miller et al., 1988). Moodie et al. (1989) observed that large larvae hatched from large eggs ingested more food than small larvae.

In addition to impacts of egg size on larval size at hatching described above, Pepin et al. (1997) showed that hatching temperature significantly influenced hatching time and larval size at hatch. Miller et al. (1995) observed that cod egg size in the field varied seasonally and this seasonal pattern corresponded with the strong ambient temperature signal. Therefore, temperature may be a significant factor influencing the size of fish eggs and larvae.

Atlantic cod (*Gadus morhua*) fisheries in the Northwest Atlantic have provided food and income for eastern Canada for over 500 years (Lear, 1998). For the last fifteen

years, however, all cod stocks have experienced dramatic declines (Baird et al., 1992; Sinclair, 1993). Many hypotheses have been developed to account for the stock decline (Myers, 1997). One of these hypotheses is an increase in mortality rate in cod early life stage which caused substantially reduced number of recruits ("Recruitment failure") (de Yong & Rose, 1993; Sinclair, 1994). Egg size and larval yolk sizes have been observed to be two important parameters in describing early life stages, which determines the growth and survival of fish larvae during endogenous feeding (Moodie et al., 1989). In this study, I evaluated the impacts of egg size and hatching temperature of cod on yolk dry weight and larval size (length and dry weight) at hatch.

## **2.2 Methods and Materials**

Thirty-two mature Atlantic cod (Northern cod stock) were maintained in a flow-through indoor tank at the Ocean Sciences Centre, Logy Bay, Newfoundland and produced eggs used in this study. Ambient seawater from Logy Bay was pumped continuously to the tank and the temperature ranged from 2 °C to 7.8 °C (Figure 2-1). Fish size ranged from 60cm to 75cm. The female to male ratio was 17:15. No food was supplied to the spawners during spawning. An egg collector was mounted on the outlet of the broodstock tank. Cod were allowed to spawn naturally. During spawning, the collector was checked and eggs were collected daily.

Fish started to spawn on April 23, 1999 and ended on June 14, 1999. A total of 34 batches were obtained during this time period, 15 of which were followed to hatch.



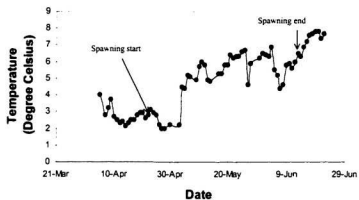


Figure 2-1. The ambient seawater temperature in the broodstock tank during the spawning

### **2.2.1 Egg Collecting and Sampling**

Eggs were collected daily and were disinfected in oxidizing liquid (5% peroxyacetic acid stabilized) for one minute, and then cleaned in filtered, UV-treated seawater for 5 to 10 minutes. Eggs were incubated in a 300-liter, flow-through, aerated incubator at different temperature with the range from 6.6 to 8.8 °C. The flow rate was 2000 ml/min. Initial density ranged from about 2500 to 4000 eggs per liter, depending on the number of the eggs collected from one spawning effort. If the density from the first collection was below this range, eggs collected over the next 24 hours were added. Random sampling of eggs took place immediately after the eggs were transferred to the incubator. If additional eggs were added the following day, samples were taken again and the mean egg diameter was determined as the average of all samples from the same incubator. Images of the sampled eggs were saved as TIF files with Image Analysis System (IAS) (Image-Pro Plus V4.1, Media Cybernetics, L. P., U.S.A) for future measurement of egg number and egg diameter. After cleaning with distilled water, eggs were dried at 69 °C for 48 hours in a pre-weighed aluminium boat covered with filter paper, cooled in a desiccator and weighed by electronic micro-balance (to nearest 0.1 µg). Egg diameters were measured from images of fertilized eggs from the 2-cell to early blastula stages.

### **2.2.2 Hatching and Larval Sampling**

The incubators were located in a temperature-controlled room. The water temperature in the incubators was allowed to vary within  $\pm 1$  °C and recorded daily. The light intensity at the surface of incubators was 32 to 79 lux (SPER Scientific light meter 840006). Filtered, UV-treated seawater was used throughout the period of incubation. Each morning, the air and water flow were stopped for 15 minutes and dead eggs (opaque eggs at the incubator bottom) were removed. Samples of randomly chosen larvae at 50% hatching were used for measuring standard length and dry weight. The larvae were first preserved in 10 % buffered formalin acetate. After 45 days, twenty larvae at 50 % hatch from each batch were used to determine larval standard length (mm) and dry weight (mg). The larval standard length was measured and yolk was removed under a dissecting microscope (to nearest 0.052mm). Preservation hardens the yolk and makes it easy to remove it from the larva in the form of a pellet (Peterson et al., 1996; Trippel, 1998). The larvae and yolks were separately placed in weak acetic acid (5%) for 2 hours to clean the formaldehyde buffer residue and then rinsed with distilled water for 30 minutes. Finally, the larvae and yolks were dried at 69°C for 48 hours, allowed to cool in a dessicator at room temperature, and weighed using an electronic micro-balance (to nearest 0.1 ug). Because preservation caused some shrinkage of length and loss of dry weight, the measured length and dry weight were adjusted to the fresh measurement by a correction factor derived from the following experiment.

### **2.2.3 Preservation Correction Experiment**

A sample of 30 larvae was taken from an incubator at 50% hatching, from which individual larvae were measured for standard length, and immediately placed in 10% buffered formalin acetate. After 45 days' preservation, the individual standard length was taken again and the result was compared with the fresh one to obtain an individual correction factor. The mean value from 30 larvae was used as a length correction factor. For the dry weight correction, four samples of 10 larvae each were taken at 50% hatching. Two of four samples were used for measuring average fresh dry weight of 10 larvae. The other two samples were preserved in 10% buffered formalin acetate for 45 days, from which the average dry weights of 10 larvae were determined after preservation as described as above. For each sample, the average dry weight was compared between fresh and preserved individuals to produce the correction factor.

### **2.2.4 Data analysis**

In this study, the incubation temperature and egg size were both subject to error. Under the assumption that they all follow normal distribution, simple linear regressions were used to describe the relationships among mean egg size, mean egg dry weight, mean larval standard length, mean incubation temperature, accumulated incubation temperature (degree-days), mean larval dry weight (with and without yolk), yolk dry weight, yolk ratio (the percentage of yolk dry weight in total larval dry weight) and days to 50% hatch. Because the larval dry weight without yolk and yolk dry weight can neither be controlled

in the study, Pearson correlation coefficient was used to test their relationship. Standardized residuals were plotted against predicted values of dependent variables to test for linearity and constancy of error variance in the regression analysis. Data were natural logarithmically transformed for regression analysis between egg diameter and dry weight variables. The Type I error was set at a 0.05 level for rejecting the null hypothesis.

## **2.3 Results**

The mean egg diameter from 15 batches of eggs ranged from 1.33mm to 1.44mm. The mean incubation temperature varied from 6.6 to 8.8 °C. The incubation time ranged from 11 to 16 days. Accumulated incubation temperature ranged from 88.8 to 105.4 degree-days (Table 2-1).

The correction experiments showed that shrinkage in length and loss in weight due to preservation was 13.5% (SE=0.46%, N=30) and 21% (SE=6.0%, N=2), respectively. At 50% hatching, the adjusted larval standard length and dry weight ranged from 4.289 to 5.081 mm and from 0.043 to 0.0519 mg, respectively. Yolk dry weight and yolk ratio varied from 0.0048 to 0.0145 mg and 9.6% to 28.3%, respectively (Table 2-1).

Mean egg diameter was significantly correlated with mean egg dry weight, larval dry weight with yolk and without yolk, incubation time, accumulated incubation temperature and larval standard length at hatching (Table 2-2, Figures 2-2, 2-3).

Table 2-1. The values of the parameters measured in the experiment.

Parameters	Minimum Value	Maximum Value
Mean Egg Size (mm)	1.33	1.44
Mean Incubation Temperature (°C)	6.6	8.8
Mean Larval Dry Weight with Yolk (mg)	0.043	0.052
Mean Larval Dry Weight without Yolk (mg)	0.035	0.047
Mean Larval Standard Length (mm)	4.289	5.081
Incubation Time (days)	11	16
Accumulated Incubation Temperature (degree-days)	88.8	105.4
Yolk Dry Weight (mg)	0.0048	0.0145
Yolk Ratio (%)	9.6	28.3

Table 2-2: Results of regression analysis between mean egg size and seven other variables for Atlantic cod

Independent Variable	Mean Egg Size (mm)		
Dependent Variable	F-value for regression model**	P-value for regression	Variance explained by the regression model
Mean Egg Dry Weight* (mg)	169.27	<0.001	92.9%
Mean Larval Dry Weight with Yolk* (mg)	21.42	<0.001	62.3%
Mean Larval Dry Weight without Yolk* (mg)	16.42	0.001	55.8%
Incubation Time (days)	25.94	<0.001	66.6%
Mean Larval Standard Length (mm)	15.68	0.002	54.7%
Yolk Dry Weight (mg)	0.06	0.811	not significant
Yolk Ratio (%)	0.398	0.539	not significant

\*Both independent and dependent variables were log transformed for the analysis.

\*\*F-value with degree of freedom of 1 (numerator) and 13 (denominator)

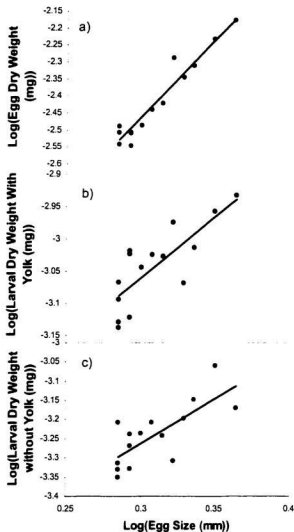


Figure 2-2: Log transformation of mean egg size (mm) and its relationship with log transformation of **a)** mean egg dry weight (mg) **b)** mean larval dry weight with yolk (mg) and **c)** mean larval dry weight without yolk (mg) for Atlantic cod



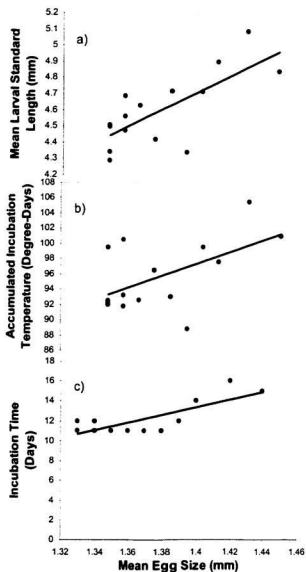


Figure 2-3. Mean egg size (mm) and its relationship with **a)** mean larval standard length (mm) **b)** accumulated incubation temperature (degree-days) and **c)** incubation time (days) in Atlantic cod

Mean egg diameter explained 92.9% of the variation in mean egg dry weight, 62.3% in mean larval dry weight with yolk, 55.8% in mean larval dry weight without yolk, 66.6% in incubation time and 54.7% larval standard length at hatching (Table 2-2). Mean egg diameter was not related to yolk dry weight or yolk ratio (Table 2-2).

Mean incubation temperature significantly affected mean larval dry weight with yolk, mean larval standard length, mean larval dry weight without yolk and incubation time (Table 2-3; Figure 2-4). Mean incubation temperature contributed 43.3% of variation in mean larval dry weight with yolk, 54.2% for mean larval dry weight without yolk, 58.5% in larval standard length and 90.6% in incubation time (Table 2-3). Mean incubation temperature was not related to mean yolk dry weight and yolk ratio (Table 2-3).

In contrast to mean egg size and mean incubation temperature, accumulated incubation temperature was significantly correlated to yolk dry weight and yolk ratio with the coefficient of determination of 0.44 and 0.57, respectively. Also there were significant relationships between accumulated incubation temperature and mean larval dry weight without yolk and mean larval standard length, respectively (Table 2-4; Figure 2-5). The accumulated incubation temperature explained 71.2% of the variation in mean larval dry weight without yolk and 41.6% in mean larval standard length. However, accumulated incubation temperature did not show any significant relationship with mean larval dry weight with yolk (Table 2-4).

Regressions between incubation time and mean larval size at hatch showed that a positive relationship existed between incubation time and mean larval dry weight

Table 2-3. Results of regression analysis between mean incubation temperature and six other variables for Atlantic cod

Independent Variable	Mean Incubation Temperature (°C)		
Dependent Variable	F-value for regression model*	P-value for regression	Variance explained by the regression model
Mean Larval Dry Weight with Yolk (mg)	9.98	0.008	43.3%
Mean Larval Dry Weight without Yolk* (mg)	18.33	0.001	54.2%
Incubation Time (days)	128.80	<0.001	90.6%
Mean Larval Standard Length (mm)	15.40	0.002	58.5%
Yolk Dry Weight (mg)	0.28	0.607	not significant
Yolk Ratio (%)	1.06	0.330	not significant

\*F-values with degree of freedom of 1 (numerator) and 13 (denominator).



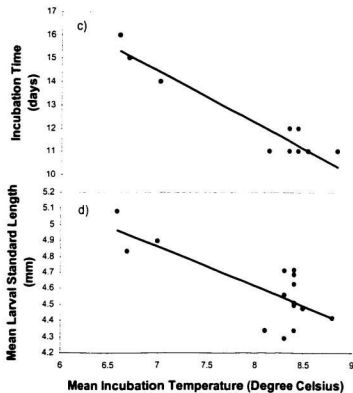


Figure 2-4 (Cont...): Mean incubation temperature (Degree Celsius) and its relationship with c) incubation time (days) and d) mean larval standard length (mm) in Atlantic cod

Table 2-4. Results of regression analysis between accumulated incubation temperature and five other variables for Atlantic cod

Independent Variable	Accumulated Incubation Temperature (degree-days)		
Dependent Variable	F-value for regression model*	P-value for regression	Variance explained by the regression model
Yolk Dry Weight (mg)	10.22	0.007	44.0%
Yolk Ratio (%)	17.57	0.001	57.5%
Mean Larval Dry Weight without Yolk (mg)	32.15	<0.001	71.2%
Mean Larval Standard Length (mm)	9.25	0.009	41.6%
Mean Larval Dry Weight with Yolk (mg)	1.01	0.333	not significant

\*F-value with degree of freedom of 1 (numerator) and 13 (denominator).

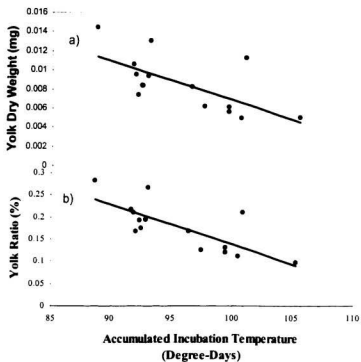


Figure 2-5: Accumulated incubation temperature (Degree Days) and its relationship with **a)** yolk dry weight (mg) and **b)** yolk ratio in Atlantic cod

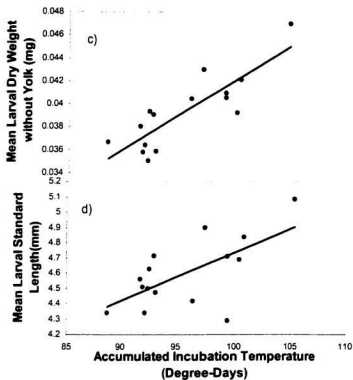


Figure 2-5 (Cont...) Accumulated incubation temperature and its relationship with **c)** mean larval dry weight without yolk (mg) and **d)** mean larval standard length (mm) for Atlantic cod



with yolk, mean larval dry weight without yolk, mean larval standard length at hatch and accumulated incubation temperature (Table 2-5; Figure 2-6). Pearson correlation between mean larval dry weight without yolk and mean yolk dry weight was significant at a 0.05 level with a correlation coefficient of  $-0.536$ . Residual plots from those regressions showed no special pattern in the regression analysis.

## **2.4 Discussion and Conclusions**

Proteins, lipids and carbohydrates are the main contributors to the dry weight of a fish egg. They make up most of the embryo and provide endogenous nutrition for the development of embryo and yolk-sac larvae for several days after hatch (Brooks et al., 1997). An increase in egg size results in increasing egg dry weight, implying a larger egg has more organic contents and can produce a larger larva or provide more nutrition for development than a smaller egg. This link was confirmed by the existence of a positive correlation between egg diameter and larval dry weight, larval standard length at hatching. A similar conclusion was reached in other studies (Miller et al., 1988; Knutsen and Tilseth, 1985; Trippel, 1998). The negative relationship between egg size and incubation time suggests that larger eggs may require a longer time to reach hatch. In contrast to results observed in another study (Trippel, 1998), this study did not detect a

Table 2-5. Results of regression analysis between incubation time and other four variables for Atlantic cod

Independent Variable	Incubation Time (days)		
	F-value for regression model*	P-value for regression	Variance explained by the regression model
Mean Larval Dry Weight with Yolk (mg)	6.82	0.022	34.4%
Mean Larval Dry Weight without Yolk (mg)	35.05	<0.001	73.0%
Mean Larval Standard Length (mm)	22.74	<0.001	63.6%
Accumulated Incubation Temperature (degree-days)	25.38	<0.001	66.1%

\*F-value with degree of freedom of 1 (numerator) and 13 (denominator).

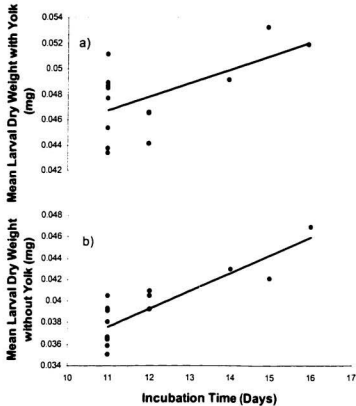


Figure 2-6: Incubation time (days) and its relationship with **a)** mean larval dry weight with yolk (mg) and **b)** mean larval dry weight without yolk (mg) for Atlantic cod

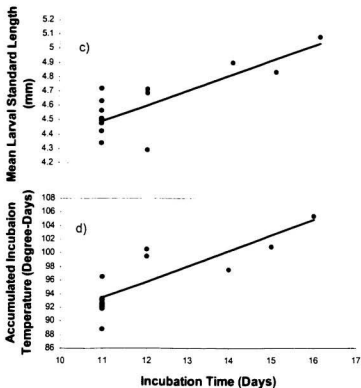


Figure 2-6 (Cont...): Incubation time (days) and its relationship with **c)** mean larval standard length (mm) and **d)** accumulated incubation temperature (Degree Days) for Atlantic cod

significant relationship between egg size and yolk size or yolk ratio. This may be due to the existence of temperature effects. Trippel's (1998) study controlled such effect by making the incubation temperature constant.

An increase in incubation temperature may increase the embryo's metabolic rate and therefore decrease the incubation time. Thus, development time was reduced at a higher incubation temperature. The negative relationships between incubation temperature and larval size at hatching suggest that high incubation temperature decreased the larval size at hatching. Thus, the longer an embryo stays inside its egg, the larger it will be at hatching.

Although the incubation temperature did not significantly affect yolk size and yolk ratio at hatching, accumulated incubation temperature was negatively correlated with these two indexes. Accumulated incubation temperature, also called thermal summation or "degree days", can be used to index the physiological age of embryos (Ojaveer, 1981; von Herbing et al., 1996), which means that higher accumulated incubation temperature resulted in the more advanced development of embryos and thus more nutrition resources (yolk) consumed by the embryos. This study showed that higher accumulated incubation temperature resulted in large larvae size and small yolk size at hatching, which supports the concept for the use of accumulated incubation temperature as a measure of physiological age. The total dry weight of egg would not change significantly during the incubation period due to the low exchanges across the egg membrane; therefore, accumulated incubation temperature was not significantly related to the larval total dry weight which is the sum of the body (increased factor) and yolk

(decreased factor). This result suggests that, like the larvae prior to exogenous feeding, the consumption of yolk results in an increase in body size of embryo during incubation. The results suggest that the total larval dry weight, including yolk at hatching, is not an indicator of development of embryo, while accumulated hatching temperature, or larvae body weight excluding yolk, or yolk at hatching, will play a significant role.

Sampling in this study followed the spawning season. Atlantic cod is a batch spawner and individual batch and physiological status of spawners have significant effects on egg and larval size at hatching (Kjesbu, 1989; Kjesbu et al., 1991; Tripple, 1998). Therefore, the effects of egg size in this study may also reflect the batch or spawner effect. However, the effect of batch or spawner could not be tested in this study because I could not identify individual spawners or batch.

The incubation temperature was not well controlled in the study, which resulted in (1) egg size and incubation temperature were highly confounded with a correlation coefficient of 0.83 and temperature and egg size were considered to be subject to error. Under the assumption that they both followed the normal distribution, the analysis in this study is appropriate and the regression model can be regarded as conditional mean function (Cook & Weisberg, 1999). (2) The data distribution for mean temperature for incubation seemed to be a bimodal (low and high clusters) pattern. An ANCOVA analysis with egg size as concomitant variable and temperature for incubation (low and high) as the treatments seems to be appropriate in the analysis. In this study, however, the egg size (the concomitant variable) was affected by the temperature (treatment) and thus covariance analysis will remove some of the effect that the treatments had on the

dependent variable. Therefore, an uncritical analysis may be badly misleading (Neter et al., 1985). The fact that temperature and egg size was subject to error violates the assumption of ANCOVA and made its application to this study unsuitable.

In summary, this study suggests that large eggs tend to have larger larvae at hatch. Larger larvae at hatch also can be achieved by low daily incubation temperatures which in turn delay hatching time and increase accumulated incubation temperature. However, this also decreases yolk size at hatch and hence shortens the period of endogenous feeding, i.e. exogenous feeding starts earlier. Although this study detected that egg size and incubation temperature both significantly influenced larval size at hatching and time for incubation, I cannot identify if such effects are caused by one or both variables because temperature and egg size was highly confounded in the study.

## **Chapter 3: Impacts of egg and larval size on growth and survival under different feeding conditions**

### **3.1 Introduction**

Growth and survival during the early life stage of fish are important in influencing fish recruitment. A small variation in growth and survival during the early life stage may result in substantial variation in fish recruitment, and subsequently in fish stock biomass (Sissenwine, 1984; Houde, 1987; Bailey & Houde, 1989; Beyer, 1989). A better understanding of larval growth and survival may help us explain the variation in fish population size. Many fish species increase weight by more than five orders of magnitude over their life span, and a  $10^3$  increment may occur in the first year of life (Werner & Gilliam, 1984; Houde, 1987). Miller et al. (1988) argued that if the standard for allocating research efforts was set by physiological time (weight stanzas) rather than calendar time, more time and energy should be spent studying the first year of life.

Mortality and growth rate in larval fish are typically high and variable (Letcher et al., 1996). Previous research has suggested that starvation and predation are two of the most important factors affecting fish larval survival (Cushing, 1976; Hunter, 1981; Leggett, 1986; Bailey & Houde, 1989). In many studies, larvae deprived of food during the switch from endogenous (yolk-sac) reserves to exogenous nutrition exhibit slow growth rates, low swimming speeds and are more vulnerable to mortality (May, 1971, 1974; Papoulias & Minnckley, 1990). These results are consistent with Hjort's (1914)



hypothesis of a “critical period”. Although Miller et al. (1988) challenged the existence of a “critical period”, they accepted that starvation during early stages can be lethal to fish larvae and further suggested that the impact of larval starvation should be closely monitored beyond the first feeding stage.

For a given fish species, the rate of growth determines the size of fish and is one of the many factors affecting larval survival (Cushing, 1975; Ware, 1975). The relationship between larval growth and survival has been explored in many studies using approaches such as evolutionary theory, simulation modeling and experimental manipulation. As a result of these studies, the theory of size-specific (stage-specific) survival (Beyer, 1989; Pepin, 1993; Pope et al., 1994; Houde, 1997; Otterlei et al., 1999) or growth-dependent mortality (Gallego & Heath, 1997) is now well established. Werner and Gilliam (1984) referred to the rate-ratio  $M/G$  ( $M$ : mortality rate and  $G$ : weight-specific growth rate) as an indicator of future population growth, i.e. minimizing the ratio of  $M/G$  during fish early life will maximize the potential for population growth. The theory has been applied in developing aquaculture strategies for juvenile Atlantic cod (*Gadus morhua*), where  $M/G$  is minimized to identify optimal habitats and release time for stock enhancement (Salvanes et al., 1994). Houde (1997) used published data on eight fish species and modeled the trends of stage-specific mortality. These models showed that for those species with  $M/G \geq 1$  the cohort biomass declined slowly ( $M/G > 1$ ) or was relatively constant ( $M/G = 1$ ) during larval stages, but for those fish species with  $M/G < 1$  (“transition size”) the cohort started to accumulate biomass. Some simulation studies show that increases in the mean and variance of fish larval growth rate tend to result in

high survival rates and can subsequently change recruitment potentials (Pepin, 1989; Rice et al., 1993; Cowan et al., 1996).

Year-class strength of fish population can be established at the egg and larval stage (Bailey & Houde, 1989). The “recruitment-failure” hypothesis considers the reduction of cohort abundance to be a result of high mortality rates and low growth rates during the early life stages (de Young & Rose, 1993; Sinclair, 1994; Campana, 1996). Cod larval survival and growth has been shown to depend on prey availability and abundance (Ottera, 1993; van der Meer & Naess, 1993; Puvanendran & Brown, 1999). Until now, almost all studies on cod larval growth and survival have been done at the level of batch, cohort or even population (Ottera, 1993; van der Meer & Naess, 1993). However, it is well known that large variation exists in larval growth and survival among individuals, and such variation can be traced to the egg stage (Blaxter & Hempel 1966). Some studies suggest that cod egg size and larval size at hatching are positively correlated in cod (Knutsen & Tilseth, 1985); therefore, egg size may also result in different characteristics in larval growth and survival (Solemdal et al., 1992).

We conducted an experiment to test the impact of egg and larval size on growth and survival of Atlantic cod larvae under two different feeding regimes. In particular, a “normal” feeding condition was contrasted with delayed feeding in larvae that differed in size at hatch.

## **3.2 Methods and Materials**

The experiment was carried out at the Ocean Sciences Center, Memorial University of Newfoundland, Canada. Eggs were collected from a broodstock tank where 17 female and 15 male Atlantic cod spawned naturally. The female cod used as broodstock ranged from 60 cm to 75 cm in total length.

### **3.2.1 Egg Collecting and Rearing**

Eggs spawned in two consecutive days were collected from an egg collector attached to the broodstock tank. The eggs were disinfected in 0.4% oxidizing liquid (5% peroxyacetic acid stabilized) for one minute and immersed in filtered, UV-treated seawater for five minutes. They were then transferred to a 25-liter flow-through bucket with aeration. A series of sieves of mesh size 1.20 mm, 1.18 mm and 1.0 mm were used to sort eggs. A sample of ten- to twenty-thousand eggs was poured on the top sieve (1.20 mm) and a fine jet of seawater was applied to allow the smaller eggs to pass through the sieves with the larger eggs retained. The eggs that were retained on the 1.20 mm sieve were classified as the big egg group, whereas those retained between the 1.18 mm and 1.0 mm sieves were defined as the small egg group. This sorting process was repeated for all collected eggs. After sieving, the two groups of eggs were placed in two, 300-liter flow-through incubators. Three 10-ml egg samples were randomly taken from each group to

determine mean diameter. An image analysis system (Image-Pro Plus V4.1, Media Cybernetics, L. P., U.S.A.) was used to measure the diameters of eggs in the samples.

Filtered, UV-treated and temperature-controlled (9-10 °C) seawater was supplied to the incubators at a rate of 2000-3000 ml min<sup>-1</sup> and eggs were monitored daily until hatch.

### **3.2.2 Larval Survival and Growth**

The larvae at 100% hatch were defined as day 0 larvae and were transferred to experimental tanks. Before transfer, the larvae were placed in a container into which seawater from the experimental tank was gradually added for acclimatization. Experimental tanks were 30- liter rectangular glass aquaria with four sides painted black. The experimental design is shown in Figure 3-1. The larvae were divided into two groups based on whether they had hatched from large or small eggs. Within each group, two feeding levels were tested. The feeding group was supplied with a prey density of 4500 prey L<sup>-1</sup> (Puvanendran & Brown 1999), whereas the delayed feeding group was given no food during the first five days (67 degree-days) and then were provided with the same prey density as in the feeding group until the end of the experiment. The time of switch from non-feeding to feeding in the delayed feeding groups was chosen to be half way to 'the point of no return' which occurs at 120--140 degree-days (15-18 days post-hatch; Gotceitas et al., 1996) and starvation is reversible. In the experiment, in order to minimize the impact of sampling on larval survival, for each feeding treatment of the

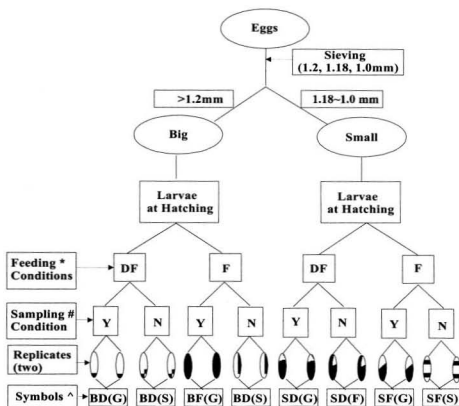


Figure 3-1. Design of experiment

\*Feeding condition: DF—Delayed feeding groups; F—Feeding groups.

#Sampling condition: Y—Sampling groups; N—Nonsampling groups.

^Symbols: BD—Larvae from big eggs and in the delayed feeding groups.

BF—Larvae from big eggs and in the feeding groups.

SD—Larvae from small eggs and in the delayed feeding groups.

SF—Larvae from small eggs and in the feeding groups.

S in parentheses: The survival group without sampling.

G in parentheses: The growth group with sampling.

different size groups, two groups were chosen, one (N and S in parentheses in Figure 3-1) for estimating survival rate without sampling and the other (Y and G in parentheses in Figure 3-1) for growth rate with samples being taken every three or four days. Each treatment was replicated twice in the experiment (Figure 3-1).

Sixteen tanks were placed in a water bath in which temperature was kept between 11 and 15°C (Figure 3-2). Each tank was aerated and supplied with running filtered seawater. General rearing protocols followed that of Puvanendran and Brown (1999). A total 1200 larvae was placed in each tank. Larvae were fed rotifers (*Brachionus plicatilis*) daily at 1000 hours and 1600 hours. Before feeding, three 10-ml water aliquots from different locations in the tank were sampled to determine average number of rotifers, and then rotifer density was adjusted to the 4500 prey L<sup>-1</sup>. A 500-ml aliquot of algae (*Isochrysis spp.*) was added twice per day just before adjustment of prey density. Light intensity at the center of tank ranged from 750 lux to 960 lux (SPER scientific light meter 840006).

### **3.2.3 Data Collection**

The experiment lasted for 21 days (average 245 degree-days). During the experiment, five (on day 0) or twenty larvae (on day 21) were randomly sampled from each tank, respectively, for measuring standard length and dry weight. On days 4, 7, 11, 14, 17, additional samples of five to ten larvae were taken from the tanks ("G" tank in the Figure 3-1) for growth measurement. The sampled larvae were preserved in 10%

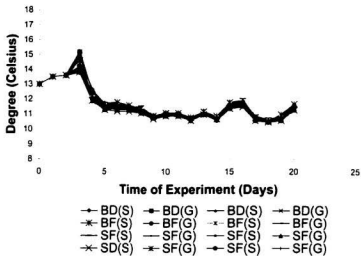


Figure 3-2. Temperature in the sixteen tanks during the experimental period. Treatment symbols as in Figure 3-1.

buffered formalin acetate. The standard length and dry weight were determined after 45 days of preservation. The standard length of each larva was measured under a dissecting microscope (to nearest 0.086 mm). Larvae were then placed in weak acetic acid for two hours to remove the formaldehyde buffer residue and rinsed in distilled water for 30 minutes (Trippel, 1998). Finally, the larvae were dried at 69 °C for 48 hours, cooled in a desiccator and weighed by an electronic microbalance (to nearest 0.1 ug). Because larvae shrink and lose dry weight during preservation, the measured length and dry weight for processed larvae were corrected to the length and weight of fresh larvae using the correction factors obtained from the following experiment.

Larvae sampled on day 0 (20 individuals), day 7 (10 individuals) and day 21(30 individuals) were measured for fresh standard length prior to preservation, and were then immediately placed in the 10% buffered formalin acetate. After 45 days, each of the 60 larvae was re-measured and compared with the fresh standard length to derive the length correction factor. For the dry weight correction, four samples (10 individuals per sample, two samples from the big larval group and two samples from the small larval group) were randomly taken on day 0. Among them, two samples (one from the big larval group and one from the small larval group) were used for estimating the means of fresh dry weight of the big larval group or small larval group. Another two were preserved in 10% buffered formalin acetate. After 45 days, the mean dry weights from different groups after preservation were calculated and compared to the corresponding fresh ones to derive the weight correction factor. The procedure for measuring dry weight was as described above. On day 21, the same procedure was repeated on six samples (10 individuals per



sample, two from mixture of the big feeding and the small feeding groups, two from small delayed feeding groups, and two from big delayed feeding groups).

Specific growth rates (SGR) of larvae were calculated, as follows:

$$SGR(SL) = \frac{Ln(L_2) - Ln(L_1)}{T_2 - T_1}$$

$$SGR(DW) = \frac{Ln(W_2) - Ln(W_1)}{T_2 - T_1}$$

Where  $L_1$  and  $L_2$  are mean length of larvae at time  $T_1$  and  $T_2$  and  $W_1$  and  $W_2$  are mean dry weight of larvae at time  $T_1$  and  $T_2$  (Puvanendran & Brown, 1999).

### 3.2.4 Data Analysis

Length and dry weight of preserved larvae were adjusted to represent those of fresh larvae to carry out the statistical analysis. A Students t-test was used for comparison of the mean lengths and dry weights between two treatment groups at the beginning of the experiment. The Tukey method (HSD) was used to carry out pairwise comparison of all treatment means of three sampling days to obtain the larval growth trends, where the experimentwise error rate was set to 0.05. The effects of egg size and feeding treatment on larval survival and growth rates were analyzed by ANOVA, where the level of significance of test was set to 0.05.

### 3.3 Results:

After sieving, the mean diameter of the big egg group was 1.38 mm (SD=0.047, N=88), and the mean diameter of the small egg group was 1.28 mm (SD=0.060, N=59). Big eggs were significantly larger than small eggs (T-test:  $t=8.89$ ,  $df=145$ ,  $p<0.001$ ).

The ANOVA analysis showed that larval size at hatch and feeding condition both significantly influenced larval survival rate (Table 3-1; Figure 3-3). The survival rate of the feeding group was higher than that of the delayed feeding group. Larvae from small eggs tended to have higher survival rate than those from big eggs for a given feeding treatment.

The correction experiment showed that the shrinkage rate for standard length and loss rate of dry weight due to 45 days preservation in 10% formalin acetate was 12% (SE=0.48%, N=60) and 23% (SE=6.4%, N=5), respectively. The mean standard length of larvae from the incubator for big egg group was 4.86 mm (SD=0.190, N=44), while the larvae from the incubator for small egg group had a mean standard length of 4.6 mm (SD=0.280, N=74). The larvae from big egg group was significantly larger than that from the small egg group ( $t=6.08$ ,  $df=116$ ,  $p<0.001$ ). At the start of the feeding experiment (day 0), the larvae in the big group tanks were significantly larger than that in the small group tanks in standard length ( $t=3.16$ ,  $t=6$ ,  $p=0.010$ ) and in dry weight ( $t=2.17$ ,  $df=6$ ,  $p=0.036$ ). However, no significant differences were found within the big egg group or within the small egg group in standard length ( $t=0.00$ ,  $df=2$ ,  $p=0.500$  within the big

Table 3-1: Results of a two-way ANOVA (feeding condition and larval length at hatching) on survival of Atlantic cod larvae during the 21-day period of the experiment.

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Sum of Squares	F	P
Larval Size at Hatching	$1.500 \times 10^{-2}$	1	$1.500 \times 10^{-2}$	18.21	0.013
Feeding Conditions	$5.600 \times 10^{-2}$	1	$5.600 \times 10^{-2}$	68.02	0.001
Interaction Term	$1.140 \times 10^{-4}$	1	$1.140 \times 10^{-4}$	0.14	0.728
Error	$3.294 \times 10^{-3}$	4	$3.294 \times 10^{-3}$		
Total	$7.442 \times 10^{-2}$	7			

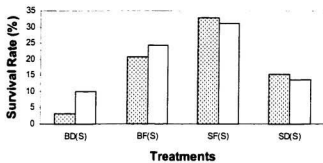


Figure 3-3. Survival rate (%) of Atlantic cod larvae reared under four treatments (with replicates). Treatment symbols as in Figure 3-1.

groups and  $t=1.06$ ,  $df=2$ ,  $p=0.200$  within the small groups) and in dry weight ( $t=0.36$ ,  $df=2$ ,  $p=0.37$  within the big groups and  $t=2.35$ ,  $df=2$ ,  $p=0.071$  within the small groups).

After five days of no food, for both the big and the small group, mean larval lengths in the delayed feeding group showed no significant difference from those in the feeding group (Table 3-2). However, mean larval dry weight showed significant differences within each group (Table 3-3), which implied the first few days of feeding mostly resulted in an increase in larval weight and not length. Between day 7 and day 11, the length of feeding larvae of the small group exceeded the length of the delayed feeding larvae of the big group (Figure 3-4). Dry weight of the feeding larvae of the small group exceeded the delayed feeding larvae of the big group on day 4 (Figure 3-5). At day 11, mean larval length and mean dry weight of the feeding groups were both significantly larger than those of delayed feeding groups. Also mean larval length and mean dry weight of the small feeding larvae were significantly larger than those of big delayed feeding larvae (Table 3-2; Table 3-3), which indicated that delayed feeding stunted the larvae. However, after sixteen days of feeding (day 21), the larvae in the big delayed feeding groups caught up with the larvae of the feeding groups, i.e. they were not significantly different from the larvae in feeding groups in length or dry weight, while the larvae in the small delayed feeding groups did not (Table 3-2, Table 3-3). The feeding larvae of the big group were consistently larger than the delayed feeding larvae of the small groups in length during the experimental period (Table 3-2).

Feeding condition also significantly influenced the specific growth rate for standard length (SL) (Table 3-4; Fig 3-6) and for dry weight (DW) (Table 3-5; Fig 3-7).

Table 3-2: Results of the Tukey analysis comparing the means of standard length of Atlantic cod larvae reared under different treatments at Day 4, 11 and 21(post hatch) of the experiment

Pair-wise Comparison of Treatment Means*	Day 4		Day 11		Day 21	
	Absolute Difference	95%SCI (L, U)#	Absolute Difference	95%SCI (L, U)#	Absolute Difference	95%SCI (L, U)#
BD vs BF	0.135	-0.21   0.48	0.675	<b>0.25</b>   <b>1.10</b>	0.930	-0.03   1.89
BD vs SF	0.255	-0.09   0.60	0.625	<b>0.20</b>   <b>1.05</b>	0.680	-0.28   1.64
BD vs SD	0.215	-0.013   0.56	0.075	-0.35   0.50	0.580	-0.38   1.54
BD vs SF	0.390	<b>0.05</b>   <b>0.73</b>	0.050	-0.37   0.47	0.250	-0.71   1.21
BF vs SD	0.350	<b>0.01</b>   <b>0.69</b>	0.600	<b>0.17</b>   <b>1.02</b>	1.510	<b>0.55</b>   <b>2.47</b>
SF vs SD	0.040	-0.28   0.40	0.550	<b>0.13</b>   <b>0.97</b>	1.260	<b>0.30</b>   <b>2.22</b>

\* BD—Large Delayed Feeding Group

BF—Large Feeding Group

SF—Small Feeding Group

SD—Small Delayed Feeding Group

# 95%SCI(L,U)—Low Limit (L) and Upper Limit (U) of 95% Simultaneous Confidence Intervals for Difference of Treatment Means

Highlighted value—Significant Difference Between Two Treatment Means, i.e. 95% SCI not including 0.

Table 3-3: Results of the Tukey analysis comparing the means of dry weight of Atlantic cod larvae reared under different treatments at Day 4, 11 and 21(post hatch) of the experiment.

Pair-wise Comparison of Treatment Means*	Day 4		Day 11		Day 21	
	Absolute Difference	95%SCI (L, U)#	Absolute Difference	95%SCI (L, U)#	Absolute Difference	95%SCI (L, U)#
BD vs BF	0.015	<b>0.009</b>	0.043	<b>0.003</b>	0.085	-0.056
		<b>0.021</b>		<b>0.084</b>		0.226
BD vs SF	0.009	<b>0.003</b>	0.058	<b>0.018</b>	0.077	-0.064
		<b>0.015</b>		<b>0.099</b>		0.218
BD vs SD	0.002	-0.004	0.009	-0.032	0.071	-0.070
		0.009		0.049		0.212
BD vs SF	0.006	-0.001	0.015	-0.026	0.008	-0.133
		0.012		0.055		0.149
BF vs SD	0.017	<b>0.011</b>	0.035	-0.006	0.156	<b>0.015</b>
		<b>0.024</b>		0.075		<b>0.297</b>
SF vs SD	0.012	<b>0.005</b>	0.049	<b>0.009</b>	0.148	<b>0.007</b>
		<b>0.018</b>		<b>0.090</b>		<b>0.289</b>

\* BD—Large Delayed Feeding Group

BF—Large Feeding Group

SF—Small Feeding Group

SD—Small Delayed Feeding Group

# 95%SCI(L,U)—Low Limit (L) and Upper Limit (U) of 95% Simultaneous Confidence Intervals for Difference of Treatment Means

Highlighted value—Significant Difference Between Two Treatment Means, i.e. 95% SCI not including 0.

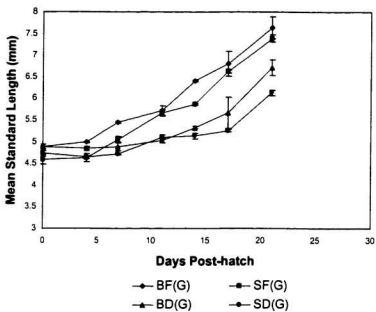


Figure 3-4. Change over time in mean ( $\pm 1$  SE from two replicates) standard length of Atlantic cod larvae reared under four treatments. Treatment symbols as in Figure 3-1.



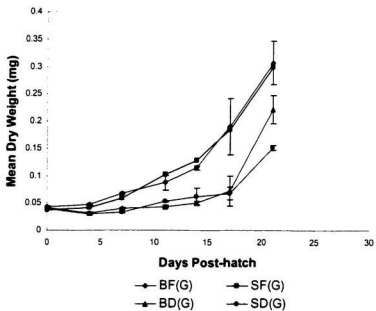


Figure 3-5. Change over time in mean ( $\pm 1$  SE from two replicates) dry weight of Atlantic cod larvae reared under four treatments. Treatment symbols as in Figure 3-1.

Table 3-4: Results of a two-way ANOVA (feeding condition and larval length at hatching) on length specific growth rate (LSGR) of Atlantic cod larvae during the 21-day period of the experiment.

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Sum of Squares	F	P
Larval Size at Hatching	$1.354 \times 10^{-4}$	1	$1.354 \times 10^{-4}$	0.34	0.59
Feeding Conditions	$1.105 \times 10^{-6}$	1	$1.105 \times 10^{-6}$	41.64	0.004
Interaction Term	$8.487 \times 10^{-6}$	1	$8.487 \times 10^{-6}$	2.61	0.18
Error	$1.301 \times 10^{-5}$	4	$3.251 \times 10^{-6}$		
Total	$1.580 \times 10^{-4}$	7			

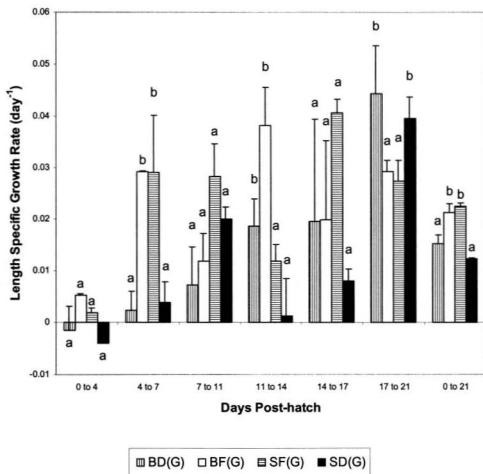


Figure 3-6. Mean ( $\pm 1$  SE from two replicates) length specific growth rate (LSGR) of Atlantic cod larvae reared under four treatments during seven different intervals. Different letters (a, b) indicate significant difference ( $P < 0.05$ ) in length specific growth rate between two treatments. Treatment identification as in Figure 3-1.

Table 3-5: Results of a two-way ANOVA (feeding condition and larval length at hatching) on weight specific growth rate (WSGR) of Atlantic cod larvae during the 21-day period of the experiment.

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Sum of Squares	F	P
Larval Size at Hatching	$4.277 \times 10^{-5}$	1	$4.277 \times 10^{-5}$	1.30	0.318
Feeding Conditions	$1.197 \times 10^{-3}$	1	$1.197 \times 10^{-3}$	36.29	0.004
Interaction Term	$1.932 \times 10^{-4}$	1	$1.932 \times 10^{-4}$	5.86	0.073
Error	$1.319 \times 10^{-4}$	4	$1.319 \times 10^{-4}$		
Total	$1.564 \times 10^{-3}$	7			

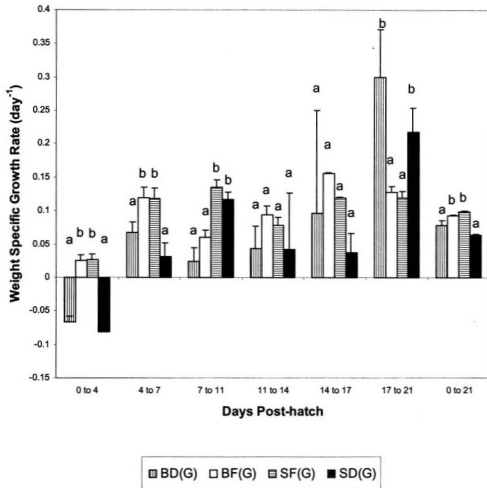


Figure 3-7. Mean ( $\pm 1$  SE from two replicates) weight specific growth rate (WSGR) of Atlantic cod larvae reared under four treatments during seven different intervals. Different letters (a, b) indicate significant differences ( $P < 0.05$ ) in weight specific growth rate between two treatments. Treatment identifications as in Figure 3-1.

However, larval size at hatch had no significant impact on specific growth rate over the experimental period (21 days) for SL and for DW. However, in the delayed feeding groups, the larvae in the big group exhibited a significantly higher specific growth rate than those in the small group for SL and for DW (Table 3-6; Table 3-7). During the non-feeding period, the larvae of the big group and the small group exhibited a decrease in mean length (Figure 3-4) and dry weight (Figure 3-5), i.e. negative growth rate (Figure 3-6; Figure 3-7), which implies that the larger larvae in each group died earlier. Alternatively, larvae may have experienced negative growth in both mean length and mean weight if they were deprived of food in the first feeding period, regardless of their size. From day 0 to day 4, the weight SGRs of feeding groups were significantly larger than those of delayed feeding groups (Table 3-8; Figure 3-7), which implied that prey availability could affect larval growth (weight) during the first 4 days post hatch at about 14 °C in cod and growth during this period mainly appeared as an increase in their dry weight. The SGRs for both length and dry weight in delayed feeding groups were significantly larger than those in the feeding group over the time period day 17 to day 21 for length and for dry weight) (Table 3-9; Table 3-10 and Figure 3-6, Figure 3-7).

### **3.5 Discussion:**

Larvae from big and small eggs showed significant differences in length at hatch, confirming the previous observation that larger eggs produce larger larvae (Chapter 2 of this thesis; Knutsen & Tilseth, 1985).

Table 3-6: Results of a one-way ANOVA (larval length at hatching) on length specific growth rate (LSGR) of Atlantic cod larvae reared in delayed feeding groups during the 21-day period of the experiment.

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Sum of Squares	F	P
Larval Size at Hatching	$1.432 \times 10^{-5}$	1	$1.432 \times 10^{-5}$	19.59	0.047
Error	$1.461 \times 10^{-6}$	2	$1.461 \times 10^{-7}$		
Total	$1.578 \times 10^{-5}$	3			

Table 3-7: Results of a one-way ANOVA (larval length at hatching) on weight specific growth rate (WSGR) of Atlantic cod larvae reared in the delayed feeding groups during the 21-day period of the experiment.

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Sum of Squares	F	P
Larval Size at Hatching	$2.992 \times 10^{-4}$	1	$2.992 \times 10^{-5}$	19.17	0.048
Error	$3.122 \times 10^{-5}$	2	$1.563 \times 10^{-5}$		
Total	$3.304 \times 10^{-4}$	3			



Table 3-8: Results of a two-way ANOVA (feeding condition and larval length at hatching) on weight specific growth rate (WSGR) of Atlantic cod larvae during the 0-4 days of the experiment.

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Sum of Squares	F	P
Larval Size at Hatching	$9.642 \times 10^{-5}$	1	$9.642 \times 10^{-5}$	0.49	0.52
Feeding Conditions	$1.976 \times 10^{-2}$	1	$1.976 \times 10^{-3}$	101.03	<0.001
Interaction Term	$1.254 \times 10^{-4}$	1	$1.254 \times 10^{-4}$	0.64	0.47
Error	$7.823 \times 10^{-4}$	4	$1.956 \times 10^{-4}$		
Total	$2.076 \times 10^{-2}$	7			

Table 3-9: Results of a two-way ANOVA (feeding condition and larval length at hatching) on length specific growth rate (LSGR) of Atlantic cod during the 17-21 days of the experiment.

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Sum of Squares	F	P
Larval Size at Hatching	$2.254 \times 10^{-5}$	1	$2.254 \times 10^{-5}$	0.49	0.523
Feeding Conditions	$3.728 \times 10^{-4}$	1	$3.728 \times 10^{-4}$	8.08	0.047
Interaction Term	$4.284 \times 10^{-6}$	1	$4.284 \times 10^{-6}$	0.09	0.776
Error	$1.846 \times 10^{-4}$	4	$4.615 \times 10^{-5}$		
Total	$5.842 \times 10^{-4}$	7			

The hatch rate for big and small eggs were similar (22% for big, 21.5% for small). Compared to an average hatch rate of about 70% for other batches of eggs (without sieving) hatched during the same period from the same broodstock (Zhao, unpublished), the hatch rates in this study were very low. This is likely due to the mechanical damage resulting from sieving the eggs. Therefore, although the sieving egg can be an effective technique in dividing the egg of different sizes, it may cause higher mortality in fish eggs. All surviving larvae were normal in appearance and there is no indication that sieving damage influenced the larval growth and survival.

Preservation causes shrinkage in larval length (Hay 1982; Trippel, 1998) and loss of weight (Peterson et al., 1996; Trippel, 1998). The variation of shrinkage in length in 2-5% formalin is larger than that in 10-30% formalin (Hay, 1982). Ten percent buffered formalin acetate was used in this study, which resulted in 12% shrinkage in length and 23% weight loss. The results of corrected standard length and dry weight of larvae-at-hatch were consistent with those measured in fresh, newly hatched cod larvae (Puvanendran & Brown, 1999), suggesting that the preservation method and correction factors used in this study were reliable.

Higher survival and growth rates in the feeding group compared to that in the delayed feeding group implies that prey availability is a critical factor during the first feeding period of cod. Even short-term deprivation of prey for the first feeding larvae, as shown in this study (five days after hatch at average 13.4 °C), can result in high mortality and low growth. This finding is consistent with observations in studies of other species (May, 1971; Storch & Juario, 1983; Papoulias & Minnckley, 1990; Yufera et al., 1993).

The results from this experiment showed that large larvae had a lower survival rate. In the wild, however, it is known that predation is one of main factors causing high larval mortality. For fish larvae, predation rate depends on encounter rate, fish larval size and swimming speed (Bailey & Batty, 1983; Letcher et al., 1996). Fast-growing larvae can attain a larger size in a shorter time compared to those growing slowly. Larvae of large sizes swim faster, search a larger volume of water for prey (Blaxter & Staines, 1971; Drost, 1987) and are less likely to be eaten by their predators (Fuiman, 1993, Williams et al, 1996). Therefore, the above result from this study may not be extended to the field study where predation mortality influences larval survival.

Cod larvae typically exhaust their yolk reserve three to five days after hatch at 5.9 °C and start exogenous feeding (Knutsen & Tilseth, 1985). The high temperature (average 13.4 °C during the first five days) in this experiment would accelerate yolk utilization. Therefore the effect of five days deprivation of food at 13.4 °C, a total of 67 °C degree-days, would be equivalent to 8-9 days at 8 °C, which is the half way to the "point of no return " (Gotceitas et al., 1996). The results confirmed that the starvation was reversible if the starved larvae can find sufficient food before they reach the half way to "point of no return".

This study showed that the cod larvae had a higher growth rate after the exogenous feeding (around day 4), which implies that the exogenous nutrition causes rapid growth in cod larvae. This suggests that larvae that start exogenous feeding earlier will grow faster and have larger size-at-age.

The growth rates of larvae in the delayed feeding groups were lower than those of larvae in the feeding group for both big and small groups except during the last part of experiment (17 to 21 days after hatch). This suggests that delayed feeding has a significant negative impact on cod larval growth. After sufficient food was provided, the larvae of the big group grew faster than those of the small group. This implies that large larvae may have a resistance to starvation and grow faster after prey is available.

The growth rate in the delayed feeding group during the 17 to 21 day period was higher than that in the feeding group. This may indicate the existence of "compensatory growth" in cod larvae (Pederson et al., 1990; van der Meeren & Naess, 1993). A few days of delayed feeding may increase the conversion efficiency of fish larvae and this increased conversion efficiency could result in an increased growth rate (May, 1971). An alternative explanation in the occurrence of compensatory growth in delayed feeding group is that selective mortality in delayed feeding groups results in the stronger and faster growth individuals surviving the starvation. When switching from no prey to abundant prey, survivors may require time for acclimatization. For the cod larvae in this study that experienced delayed feeding for five days (67 degree-days), the time required for acclimatization appears to be 10 to 13 days, after which the larvae exhibit compensatory growth.

The experiment ended on day 21 (average 245 degree-days) when the growth rate of larvae in the delayed feeding group exceeded that of the feeding groups. The question of whether such a growth pattern would be maintained beyond the experimental period remains unanswered.

Compared to the results of the first three weeks in Puvanendran and Brown's (1999) study, larval survival and growth in the feeding groups of the present study is similar to their high prey density ( $\geq 4000$  prey  $L^{-1}$ ) treatment groups, while delayed feeding groups are comparable to their low prey density ( $\leq 2000$  prey  $L^{-1}$ ) groups. This implies that delayed initial feeding may have the same effects on larval growth and survival as insufficient food supplies.

Maintaining constant temperature during larval culture can minimize the impacts of temperature fluctuation on larval growth and survival, given that temperature is a very important factor influencing larval growth and survival (Brett, 1979; Campana & Hurley, 1989). The fluctuating temperatures that occurred during the first few days of the experiment may have had different impacts on the different groups of size of larvae from different egg groups. The temperature differences, however, were relatively small (11-15 °C) and of short duration (three days) which is unlikely to have significantly affected larval growth and survival in this study.

In summary, my findings suggest that large eggs tend to produce large larvae. After hatch, survival and growth of larvae are affected by both larval size and prey availability. Cod larvae mainly increased their weight during the first few days' growth. Large larvae have a greater ability to recover from short-term deprivation of food. Delayed feeding can cause high mortality, but it may also result in compensatory growth response in the survivors after a period of good feeding.

## **Chapter 4: Summary and conclusion**

This study confirmed that egg size and larval size are closely related. Low incubation temperature extended the hatching time but resulted in large larvae at hatching. However, large size at hatch due to prolonged incubation period resulted in decreased yolk size. An increase in accumulated incubation temperature increased the larval length at hatch but decreased the yolk size and thus may index the larval physiological age. The fact that larval body size at hatching was negatively correlated to yolk size at hatching implies that embryo's growth consumes the yolk and "endogenous feeding" occurs before the larva is hatched.

The fact that egg size and temperature are both highly confounding and subject to error in this study makes it impossible to carry out a multifactor analysis on their effects. Future studies should use a factorial design to test the effects of egg size and temperature on larval and yolk size at hatching.

The feeding condition and larval size at hatching both significantly influenced the larval growth and survival. Delayed first feeding caused higher mortality in cod larvae and small larvae may survive better than large larvae in the absence of predation. Although delayed first feeding resulted in higher mortality, starvation is reversible if the larvae can find the suitable and sufficient food before they reach the "point of no return" and survivors can exhibit "compensatory growth" after resuming feeding for a short time.

The result that large larvae are superior to small larvae for growth is only evident under conditions of deficiencies in prey availability, which suggests that “The bigger, the better” theory is suitable for cod larvae living under unfavourable environmental conditions. Larval viability was influenced by size and the first feeding condition. Therefore, in future studies of larval survival, growth and thus recruitment it is essential to include information on prey availability during the first feeding and larval size at hatching as well.

In this study, I demonstrated the relationship between egg and yolk size, but I did not test the effects of yolk size at hatching on larval growth and survival. Yolk is the only nutrition resource available to a larva before exogenous feeding begins and it helps to complete morphological development of the larval digestive system necessary to process the external food (Bisbal & Bengtson, 1995; Gisbert et al., 1998). Therefore, yolk quality (yolk size or ratio) may be one of factors causing mortality during early larval life and the usefulness of yolk indices (chemical components, size, ratio) as predictors of larval survival and growth should be explored in the future studies.

The lab experiment on larval growth and survival in Chapter 3 ended with the observation of compensatory growth in the delayed feeding condition. Is this pattern going to continue beyond the experimental period and how long can the larvae in delayed first feeding groups maintain this “compensatory growth”? Will the time of compensatory growth be long enough for the larvae in the delayed feeding groups to catch up with those in the feeding groups? All these questions remain unanswered and will be explored in a future study.



In this study, I used the mean of the variables, such as mean of egg size, larval size and temperature, growth rate, etc., to carry out the analysis. Pepin (1989) demonstrated that variation in the variance of the growth parameters significantly influence survival in fish larvae. The impacts of variation in variance in these parameters should also be explored.

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